P3442

Board Number: B733

Programmable viscoelastic matrices from artificial proteins.

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Extracellular matrix compliance influences cellular adhesion and migration, proliferation and apoptosis, and differentiation. Much of our current knowledge of the effects of substrate stiffness on cellular behavior is based on elastic substrates, in particular cross-linked polyacrylamide hydrogels. Biological tissues, however, are viscoelastic and exhibit stress relaxation and energy dissipation on physiologically relevant timescales. While emerging evidence suggests that these physical properties also influence cellular behavior, materials in which viscoelasticity can be precisely engineered are currently lacking. Here, we describe programmable hydrogel matrices assembled from artificial recombinant proteins designed to be cross-linked by covalent bonds involving cysteine residues, by association of helical domains as coiled coils, or by both mechanisms. Using these proteins, we construct chemical, physical, and chemical-physical hydrogel networks that deform elastically or viscoelastically depending on the type of cross-linking (Dooling *et al.*, *Adv. Mater.*, 2016, 28, 4651–4657). In viscoelastic networks, the amount of stress relaxation is tuned by controlling the ratio of physical cross-linking to chemical cross-linking, and the timescale for stress relaxation is tuned over five orders of magnitude by single point mutations to the coiled-coil physical cross-linking domain (Dooling and Tirrell, *ACS Cent. Sci.*, 2016, 2, 812–819). The genetic engineering approach also allows biological activity to be encoded directly within the protein sequence in the form of cell-adhesive domains and proteolytic cleavage sites. The capacity to program the viscoelasticity and biological activity of hydrogel matrices is anticipated to have applications in studying and engineering cell-matrix interactions.

Chaperones, Protein Folding, and Quality Control 2

P3443

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Subcellular localization, uptake and dynamics of inorganic polyphosphate in mammalian cells.

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Inorganic polyphosphate (polyP), a linear chain of 3 to over 100 orthophosphates, is a highly conserved and universal molecule found in all species tested. Yet, unlike in bacteria where its biosynthetic pathway has been well characterized, little is known about polyP metabolism in mammals. This is largely due to a lack of feasible tools for detection and manipulation. Here we utilize a specific probe called MBP- *Ec*PPX$_c$ (a fusion protein of maltose binding protein and the polyP binding domain of *E. coli* exopolyphosphatase) to monitor subcellular localization and dynamics of polyP by immunofluorescence. In several cell culture models including HeLa cells and primary human fibroblasts, we observed endogenous polyP in the nucleus (with enrichment in the nucleolus), plasma membrane and distinct foci in the cytoplasm. By using exogenously applied polyP$_{300}$ chains labeled with Alexa Fluor 647 (polyP$_{300}$ - AF647), we furthermore discovered that cells are able to rapidly take up polyP from the environment through endocytosis. This finding enables us to increase cellular polyP level under normal culture condition, and offers the unique opportunity to reveal the physiological function of polyP in mammalian cells. Finally, we observed a massive increase of polyP in response to hypochlorous acid (HOCl) treatment, the first known induction of polyP synthesis in mammalian cells. Taken together, we have